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PRIMER NOTE

Polymorphic microsatellite markers for inferring diversity in wild and domesticated sugar beet (*Beta vulgaris*)

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Abstract

Eight microsatellite loci were characterized within two cultivated beet (*Beta vulgaris* ssp. *vulgaris*) accessions and one accession of the wild progenitor of domesticated sugar beet, *Beta vulgaris* ssp. *maritima*. Allele diversity was high, yielding two to 11 alleles per locus. Polymorphism information content (PIC) values obtained for these eight loci where also high and indicate the highly informative nature of the microsatellites presented here. These described markers add to a small set of publicly available microsatellite markers for beet and will be instrumental in identifying patterns of genetic diversity and origins of domestication.

Keywords: *Beta vulgaris*, genetic diversity, SSR

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The use of genetic markers for inferring patterns of genetic differentiation has been an important tool in both agricultural and evolutionary studies. The comparison of quantitative trait variation and neutral marker variation across different natural populations of wild *Beta vulgaris* ssp. *maritima* may provide insight into the pattern and scale of natural selection. These data are useful for sampling diversity for crop improvement but also in understanding the process of domestication from wild germplasm. Currently, there is only a limited set of publicly available microsatellite markers for use in beet (*Beta vulgaris*, L.). The objectives of this work were to identify additional simple sequence repeat (SSR) markers from genomic libraries of sugar beet and test their efficacy in differentiating accessions of wild and cultivated beet.

Several grams of young leaf tissue from 'FC709–2' (Panella 1999) plants were pooled for bulk extraction of genomic DNA using a modification of the method of Gepts *et al*. (1992). After extraction, the crude DNA was purified by caesium chloride density gradient centrifugation. A small insert genomic DNA library was developed following a standard protocol (Rafalski *et al*. 1996). Repeat-containing fragments were selected by hybridization with a mixture of biotinylated oligonucleotides $(CA)_{15}$ $(GA)_{15}$ and $(CT)_{15}$, recovered by magnetic beads linked to streptavidin, and cloned into lambda ZAP II™ phagemid vector (Stratagene, La Jolla, CA). Repeat positive clones were identified by two rounds of screening polymerase chain reactions (PCRs), each using a repeat primer in combination with one of the vector primers adjacent to the multiple cloning site (i.e. T7 or T3 promoter primers). Positive clones were sequenced on an automated DNA sequencer (Model 377, Applied Biosystems, Foster City, CA) and primers were designed manually for regions flanking the core repeat motif. Primers were 20 bp in length, had a fairly high melting temperature (> 58 °C), and amplified DNA fragments between 120 and 300 bp in length (Table 1).

Accessions used in genotypic assessments were drawn from three different sources. PI 590766 'FC 712' (Hecker & Ruppel 1986) is a heterogeneous, broad-based, sugar beet germplasm (*B.* vs. ssp. *vulgaris*). PI 604031 is a wild sea beet (*B.* vs. ssp. *maritima*), a wild relative and probable progenitor of cultivated beet, collected from a population along the southern coast of Ireland. PI 632960 'W300C' was released in 1972 from the University of Wisconsin table beet breeding program. Polymorphism data were derived from a total 73 individuals representing, sugar beet (n = 17), sea beet (n = 41), and table beet (n = 15). PCR was carried out in a total volume of 10 μL containing: 1.5 units Fermentas *Taq* DNA

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Table 1 Primer sequence information and summary statistics for the microsatellite loci analysed. Allele size range and number (#) of alleles observed, expected heterozygosity (H_E) and polymorphism information content value (PIC value) are derived from totals taken over the whole study. GenBank accession #s are also listed for a representative allele at each locus

Primer	Sequence	Number of alleles observed	Allele size range (bp)	Core Motif	H_E	PIC Value	GenBank Accession number
SB04	Forward: 5′-ACC GAT CAC CAA TTC ACC AT-3′ Reverse: 5′-GTT TTG TTT TGG GCG AAA TG-3′	6	172–187	$(GGA)_4$ $(GTT)_8$	0.802	0.771	AY499541
SB06	Forward: 5′-AAA TTT TCG CCA CCA CTG TC-3′ Reverse: 5′-ACC AAA GAT CGA GCG AAG AA-3′	8	144–168	$(CTT)_6$	0.621	0.682	AY499542
SB07	Forward: 5′-TGT GGA TGC GCT TTC TTT TC-3′ Reverse: 5′-ACT CCA CCC ATC CAC ATC AT-3′	6	246–276	$(TC)_{10}$	0.771	0.724	AY499543
SB09	Forward: 5′-TGC ATA AAA CCC CCA ACA AT-3′ Reverse: 5′-AGG GCA ACT TTG TTT TGT GG-3′	2	129–132	$(CAT)_7$	0.440	0.354	AY499544
SB10	Forward: 5′-TTC GTC CCT TGA TTG TGT CA-3′ Reverse: 5′-GAG ATT GGG GAT CAC TCT GC-3′	2	193–197	$(GAT)_6$	0.436	0.325	AY499545
SB11	Forward: 5′-CGA GGG GTA AAA CCA GAC AA-3′ Reverse: 5′-GGT TCT GAA ATT TGG GGG TT-3′	5	169–177	$(GA)_8$	0.456	0.682	AY499546
SB13	Forward: 5′-ACA GCA AGA TCA GAG CCG TT-3′ Reverse: 5′-TGG ACC CAC CAT TTA CAT CA-3′	3	126–132	$(GAT)_9$	0.517	0.764	AY499547
SB15	Forward: 5′-CAC CCA GCC TAT CTC TCG AC-3′ Reverse: 5′-GTG GTG GGC AGT TTT AGG AA-3′	11	135–165	$(CT)_8$	0.704	0.796	AY499548

Polymerase, 1X Reaction Buffer [50 mM KCl,10 mM Tris-HCl (pH 8.8) and 0.08% Nonidet P40] (Fermentas, Inc. Hanover, MD), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.1 pM of each primer — forward primers labelled with IRDye™ 700 or IRDye™ 800 (MWG Biotech, Inc., High Point, NC) and unlabelled reverse primers and 0.1 ng of template DNA prepared as above. PCR conditions were as follows: (1) 95 °C for 5min; (2) 95 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s for 35 cycles; (3) 72 °C for 1min. PCR products were electrophoresed in a 6.5% KB^{Plus} Gel Matrix on a LI-COR 4200 sequencer (Licor, Lincoln, NB). Scanned TIFF images of each gel were used for fragment sizing and allele assignments using SAGA GT™ software (LI-COR Biosciences, Lincoln, NE). Genotypic data matrices were analysed using GDA software (Lewis & Zaykin 2001) and FSTAT (Goudet 1995) for estimates of heterozygosity, gene diversity and genetic differentiation among accessions.

A total of 43 alleles were scored across all three accessions (Table 1) Each SSR locus produced from two to 11 alleles and most of these alleles showed clear separation without substantial stutter. Each of the eight loci displayed high levels of heterozygosity and moderate to high PIC values, especially in the wild germplasm. Null allele estimation was not possible using deviations from Hardy–Weinberg expectations because samples were from mixed sources and were not expected to be in equilibrium. Genetic differentiation as measured by F_{ST} among the three accessions tested was quite high (F_{ST} = 0.497 with a bootstrapped 95% confidence interval of ±0.184).

In sugar beet, microsatellites have been used to measure genetic diversity (Mörchen *et al*. 2001), but also have been an excellent marker for looking at gene flow, within and between populations and among wild, weedy and cultivated populations (Raybould *et al*. 1998; Desplanque *et al*. 1999; Laporte *et al*. 2001; Vivard *et al*. 2002). In addition, the comparison of neutral variation as assessed from microsatellite diversity and quantitative trait variation has the potential of estimating the spatial scale of selection in natural populations. Although this study describes only eight loci, the amount of information is quite high and substantially adds to the small set of 15 microsatellite primers currently in the public domain. The eight additional microsatellite markers described in this report will be instrumental in allowing more studies examining the spatial scale of popu-lation diversity in wild accessions as well as managing the diversity within core collections of this crop.

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